REMARKS

In the Office Action, an election of species requirement has been made; the specification has been objected; claims 1-4 are rejected under 35 U.S.C. § 112, second paragraph; claims 1-4 are rejected under 35 U.S.C. § 101; claims 1-6 are rejected under 35 U.S.C. § 112, first paragraph; claims 5 and 6 are rejected under 35 U.S.C. § 102; and claims 1-4 are rejected under 35 U.S.C. § 103. Claims 7-12 have been newly added; claims 1-6 have been cancelled; and the specification has been amended. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with Markings to Show Changes Made**." Applicants respectfully submit that the rejections have been overcome in view of the amendments and for the reasons set forth below.

At the outset, the Patent Office has withdrawn the previously asserted restriction requirement. However, the Patent Office now requires an election of species with respect to the bromelain protease as characterized in claim 1. In response, Applicants elect without traverse the species with respect to the bromelain protease as characterized in Part A of claim 1. This species is readable on each of the pending and newly added claims 7-12. Applicants note for the record that they reserve the right to file a divisional application, if necessary, with respect to the non-elected claimed subject matter. Therefore, Applicants believe that they have been responsive to the Patent Office's election of species requirement.

The specification has been objected. With respect to the title, this has been changed to replace the term "bromelaine" with the term "bromelain." With respect to the publication quotation, the specification has been amended to change the first author identified from Eckert et al. to Harrach et al.

With respect to the use of the term "bromelaine" throughout the text of the specification, Applicants believe that one skilled in the art would readily recognize that this term is interchangeable in meaning with the term "bromelain." In view of same, Applicants have added text in the specification on page 1 indicating same. Therefore, Applicants believe that they have been responsive to the Patent Office's comments regarding same.

Further, the specification has been amended in response to the Patent Office's comments regarding alleged spelling, grammar and typographical errors. Applicants believe that no new matter has been added by way of the amendments discussed above. Therefore, Applicants

believe that the amendments to the specification have been responsive to the Patent Office's objections regarding same.

In the Office Action, claims 1-4 are rejected under 35 U.S.C. § 112, second paragraph. At the outset, claims 1-4 have been cancelled thus rendering moot this rejection with respect to same. Further, claims 7-12 have been newly added. Applicants believe that these claims comply with 35 U.S.C. § 112, second paragraph.

Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 1-4 are rejected under 35 U.S.C. § 101. As previously discussed, claims 1-4 have been cancelled thus rendering this rejection moot with respect to same. Further, claims 7-12 have been newly added. Applicants believe that claims 7-12 comply with 35 U.S.C. § 101.

Accordingly, Applicants respectfully request that this rejection be withdrawn.

In the Office Action, claims 1-6 have been rejected under 35 U.S.C. § 112, first paragraph. The Patent Office alleges that claim 6 does not meet the written restriction requirement; and that claims 1-6 are not enabled by the specification.

At the outset, claims 1-6 have been cancelled thus rendering moot this rejection with respect to same. Further, Applicants have added claims 7-12 and respectfully submit that these claims comply with the requirements pursuant to 35 U.S.C. § 112, first paragraph.

Although Applicants do not agree with the enablement rejection, Applicants have amended the specification, in the spirit of cooperation, to include a further description of how the bromelain protease of the claimed invention can be isolated, purified and characterized based on the Eckert and Maurer publications that were incorporated by reference on page 5 of the originally-filed specification. In view of same, Applicants believe that one skilled in the art would be enabled to make and/or use the present invention.

Applicants are also submitting herewith an affidavit from Mr. Robert M. Barrett, the undersigned attorney of record, attached hereto as Exhibit A. Applicants submit that no new matter has been added by way of this amendment as supported by the *Barrett* Affidavit. Therefore, Applicants believe that the claimed invention satisfies the enablement requirement.

With respect to the enablement and written description requirement regarding the claim term "a recombinant bromelain protease" (See, Claim 12), Applicants believe that these rejections are not proper. At the outset, the Patent Office alleges that the specification fails to disclose cloning in sequencing genes that encode the protease as required by the claimed

invention. To the contrary, the specification discloses that a recombinant protein can be provided in a conventional manner. See, specification, page 5, lines 2-3. In this regard, Applicants believe that this information is entirely within the grasp and understanding of one skilled. Thus, undue experimentation would not be required to make and/or use a recombinant bromelain protease, and further one skilled in the art would recognize that Applicants had possession of the claimed recombinant bromelain protease at the time the application was filed. Therefore, Applicants believe that the claimed invention satisfies the written description and enablement requirements.

Accordingly, Applicants respectfully request that the rejection of the claimed invention under 35 U.S.C. § 112, first paragraph be withdrawn.

In the Office Action, claims 5-6 are rejected under 35 U.S.C. § 102 in view of Harrach et al., Isolation and Partial Characterization of Basic Proteases from Stem Bromelain, Journal of Protein Chemistry, 1995, Vol. 14, pp. 41-52 ("*Harrach*"). The Patent Office essentially asserts that *Harrach* discloses each and every feature as defined by claims 5 and 6.

At the outset, claims 5 and 6 have been cancelled as previously discussed. Thus, the anticipation rejection with respect to same has been rendered moot and should be withdrawn.

Further, Applicants believe that *Harrach* fails to anticipate newly added claims 7-12. Claims 7-10 relate to a method of inhibiting blood coagulation by administering a bromelain protease as required by the claimed invention. Indeed, similar subject matter as claimed in originally-filed claims 1-4 was not rejected for anticipation purposes in view of *Harrach*. Therefore, Applicants believe that *Harrach* fails to disclose the claimed invention as defined by newly added claims 7-10 for substantially the same reasons as it fails to disclose the claimed invention as defined by claims 1-4.

With respect to newly added claims 11-12, claim 11 is the sole independent claim. In this regard, claim 11 recites a medicament for inhibiting blood coagulation that includes a composition that includes a bromelain protease as claimed. Applicants believe that patentable weight should be given to the preamble as claimed. In this regard, the medicament is for inhibiting blood coagulation. Indeed, the Patent Office admits *Harrach* fails to disclose that F4 has an inhibitory effect on coagulation of blood. Based on at least this reason, Applicants believe that *Harrach* fails to disclose the claimed invention as defined in newly added claims 11-12.

Accordingly, Applicants respectfully request that the anticipation rejection be withdrawn.

In the Office Action, claims 1-4 are rejected under 35 U.S.C. § 103 in view of *Harrach* and further in view of Taussig et al., Bromelain, the Enzyme Complex of Pineapple (*Ananas comosus*) and its clinical application, an update, Journal of Ethnopharmacology, 1988, Vol. 22, pp. 191-203 ("*Taussig*"). The Patent Office primarily relies on *Harrach* and thus relies on *Taussig* to remedy the deficiencies of *Harrach*.

At the outset, claims 1-4 have been cancelled as previously discussed. Thus, this rejection with respect to same has been rendered moot.

As previously discussed, claims 7-12 have been newly added. Applicants believe that *Harrach* is clearly deficient with respect to the claimed invention for substantially the same reasons as previously discussed. Again, the Patent Office even admits *Harrach* fails to disclose that F4 has an inhibitory effect on coagulation of blood.

Even if combinable, Applicants do not believe that *Taussig* can be relied on solely to remedy the deficiencies of *Harrach*. Nowhere does the *Taussig* publication disclose or suggest that the bromelain protease as required by the claimed invention can be administered to effectively inhibit blood coagulation, such as a medicament thereof. Indeed, the Patent Office merely relies on an "expectation of success" to justify the modification of *Harrach* in view of *Taussig*. See, Office Action, page 13.

What the Patent Office appears to have done is to apply hindsight reasoning to justify this rejection. Of course, this is improper. Again, the claimed invention relates a specific bromelain protease that has been found to be effective for inhibiting blood coagulation. Clearly, the cited art fails to disclose or suggest such features as required by the claimed invention. Therefore, Applicants do not believe that one skilled in the art would be so inclined to modify the cited art to arrive at the claimed invention.

Based on at least these noted reasons, Applicants believe that the cited art is deficient with respect to the claimed invention. Therefore, Applicants respectfully submit that the cited art, even if combinable, fails to render obvious the claimed invention.

Accordingly, Applicants respectfully request that the obviousness rejection be withdrawn.

For the foregoing reasons, Applicants respectfully submit that the present application is now in condition for allowance and earnestly solicit reconsideration of same.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please replace the Title on page 1 with the following Title paragraph:

USE OF BROMELAIN~~E~~ PROTEASES FOR INHIBITING BLOOD COAGULATION

Please substitute the paragraph beginning on page 1 at line 13 with the following rewritten paragraph:

Bromelaine is a mixture of quite different proteins that may be isolated from plants of the family Bromeliaceae, the exact composition of which could so far not yet be completely characterized due to the complexity and variety of the components contained therein. It could, however, be shown that bromelaine contains different phosphatases, cellulases, glycosidases, cysteine proteases and the peptide inhibitors thereof, as well as additional not yet more closely identified components. The material and quantitative composition of bromelaine, however, varies in response to the origin and the isolation procedure from the respective source, so that different methods for isolating the raw product, for standardizing the same as well as for purifying specific components contained therein, have been developed. As used herein, the term "bromelaine" or other like terms is meant to be interchangeable with term "bromelain."

Please substitute the paragraph beginning on page 2 at line 18 with the following rewritten paragraph:

Due to the development in the field of purification techniques it has been possible to isolate and partially also characterize additional components from the bromelaine mixture. Thus, it was disclosed by ~~Eckert~~ Harrach et al. in The Journal of Protein Chemistry **14** (1995), 41-52, that bromelaine contains at least 8 basic proteases, which could be fractioned by means of FPLC-cation exchange-chromatography. Also, the existence of two forms of acidic proteases could be shown (Maurer et al., Journal of Protein Chemistry **17** (1998), 351-361).

Please replace the paragraphs beginning on page 3 at line 17 with the following rewritten paragraphs:

It has been shown that especially the production of plasmin is stimulated by the bromelaine proteases, while the formation of fibrin and the adhesion of thrombocytes on

endothelium cells – all of which are processes playing a significant role in blood coagulation – are inhibited.

In a preferred embodiment of the invention especially basic proteases are applied for the indicated purpose, preferably the bromelaine proteases obtained as fractions F4, F5 or, more preferably, F9 in accordance with the method described by ~~Eckert~~ Harrach et al. in the Journal of Protein Chemistry **14** (1995), 41-52.

Please replace the paragraph beginning on page 4 at line 28 with the following rewritten paragraph:

The proteases can be isolated in accordance with conventional methods. Especially a purification as indicated by ~~Eckert~~ Harrach et al. in the Journal of Protein Chemistry **14** (1995), 41-52 and by Maurer et al. in the Journal of Protein Chemistry **17** (1998), can be applied. Upon purification, said proteases can be initially sequenced, and the corresponding gene can be isolated from the genome of e.g. the pineapple by means of molecular-biological methods. By means of molecular-biological methods a recombinant protein can then be provided in a conventional manner.

Please replace the paragraph beginning on page 5 at line 8 with the following rewritten paragraph:

The proteases used in the present invention, especially the basic proteases, are isolated according to ~~Eckert~~ Harrach et al., The Journal of Protein Chemistry **14** (1995), 41-52 and according to Maurer et al., The Journal of Protein Chemistry **17** (1998). The contents of said publications are herewith entirely included in the contents of disclosure of the present application.

As disclosed in Harrach (1995), crude bromelain extracts from pineapple stems (*Ananas comosus*) were fractionated by two-step FPLC-cation-exchange chromatography. At least eight basic proteolytically active components were detected. The two main components F4 and F5 together with the most active proteinase fraction F9 were characterized by SDS-PAGE, mass spectroscopy, multizonal cathodal electrophoresis, partial amino acid sequence, and monosaccharide composition analysis. F9 amounts included about 2% of the total protein and had a 15 times higher specific activity against the substrate L-pyroglutamyl-*l*-phenylanalyl-*l*-

leucine-*p*-nitroanilide (PFLNA) than the main component F4. The molecular masses of F4, F5, and F9 included 24,397, 24,472, and 23,427, respectively, as determined by mass spectroscopy. Partial N-terminal amino acid sequence analysis (20 amino acids) revealed that F9 differs from the determined sequence of F4 and F5 by an exchange at position 10 (tyrosine serine) and position 20 (asparagine glycine). F4 and F5 contained fucose, N-acetylglucosamine, xylose, and mannose in ratio of 1.0:2.0:1.0:2.0, wherein 50% of the proteins appeared to be glycosylated. F9 was found to be unglycosylated. Polyclonal antibodies (IgG) against F9 detected F4 and F5 with tenfold reduced reactivity. The *p*H optimum of F4 and F5 was between *p*H 4.0 and 4.5. For F9, the *p*H optimum was close to neutral *p*H. The kinetic parameters for PFLNA hydrolysis were similar for F4 (K_m 2.30 mM, k_{cat} 0.87 sec^{-1}) and F5 (K_m 2.42 mM, k_{cat} 0.68 sec^{-1}), and differed from F9 (K_m 0.40 mM, k_{cat} 3.94 sec^{-1}).

As disclosed in Maurer (1998), two forms of an acidic bromelain proteinase isolated from crude bromelain, an extract from pineapple stem, were found by a two-step FPLC purification procedure. The basic main components were removed by cation exchange chromatography and the breakthrough fraction was further resolved by anion exchange chromatography into 15 protein fractions, only two of which, called SBA/a and SBA/b, were proteolytically active. These components were characterized by electrospray mass spectroscopy (ESMS), isoelectric focusing, N-terminal amino acid sequence analysis, monosaccharide analysis, and enzymatic parameters. The molecular masses of SBA/a and SBA/b were determined by ESMS to be 23,550 and 23,560, respectively. The isoelectric points (pI) of the two bands of SBA/a were 4.8 and 4.9. SBA/b focused as a single band at pI = 4.8. Partial N-terminal amino acid sequences (11 residues) were identical to SBA/a and SBA/b and identical with those of stem bromelain (e.g., the basic main proteinase of the pineapple stem) and fruit bromelain (e.g., the acidic main proteinase of the pineapple fruit). Both components are highly glycosylated. Hydrolysis of SBA/a yielded about twofold more monosaccharide per protein than SBA/b. The comparison of the catalytic properties of SBA/a with those of SBA/b revealed no relevant differences in the hydrolysis of three peptidyl-NH-Mec substrates and in the inhibition profiles using chicken cystatin and E-64, thus indicating that these components can be considered as two forms of a single enzyme. Both forms are not inhibited very much by chicken cystatin and are slowly inactivated by E-64 and thus are nontypical cysteine proteinases of the papain superfamily.

Please replace the paragraph beginning on page 9 at line 22 with the following rewritten paragraph:

Thrombocytes isolated from human whole blood are marked with the fluorescence dye 2,7-bis-(2-carboxyethyl)-5,6-carboxyfluoresceinacetoxymethylester. Permanent BKEz-7 bovine aorta cells (11th-22nd passage) are pipetted into a 96 microtiter plate with 60,000 cells per recess and are incubated ~~over night~~ overnight. For the thrombocytes-endothelium cell-adhesion-assay $5x10^7$ thrombocytes after an incubation time of 15 min. at 37°C are optimal. The removal of the non-bonded thrombocytes is effected by washing the cells with KRB-buffer (Krebs-Ringer-bicarbonate buffer with 5.6 mMol Glucose + 1 % BSA) twice.

In the Claims:

Claims 1-6 have been cancelled without prejudice or disclaimer.

Claims 7-12 have been newly added.